

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Jon A. Wolff,)
Vladimir S. Trubetskoy,)
Sean D. Monahan, James E. Hagstrom,)
Paul M. Slattum, Vladimir G. Budker,)
Aaron G. Loomis)

Serial No.: 09/328,975)

Filed: 6/9/99)

Group Art Unit: 1632)

Examiner: Richard Schnizer

For: Charge Reversal of Polyion Complexes

DECLARATION UNDER 37 C.F.R. §1.132

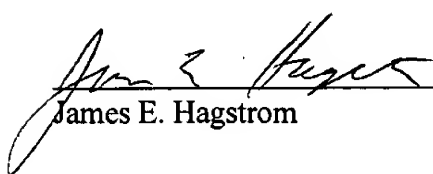
Assistant Commissioner for Patents
Washington, DC 20231

Dear Sir:

I, James E. Hagstrom, hereby declare as follows:

1. I am an inventor of the captioned application.
2. Applicants' process was conceived prior to the effective date of the Office Action prior art references.
3. We developed our recharging process with due diligence from conception to the filing of our application.
4. Photocopies of my personal laboratory notebook pages showing nucleic acids recharged with histone and then re-recharged with liposomes dated March and June, 1994 accompany this Declaration.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.


James E. Hagstrom
Date 9/7/01

3/28/

RAT Myotube Transfection

↓ ALL DNA in pH 8.5 Tris

- ¹/₂> 5 μ g pBSLucX 5 μ g/plate
³/₄> DNA + Lipofectin 2.5 μ l/plate
⁵/₆> DNA + Lipofectin 1.5 μ l/plate
⁷/₈> DNA + Lipo (2.5 μ l/plate) + NLS-H1 (0.5 μ g/plate)^(1.25)
⁹/₁₀> DNA + Lipo (2.5 μ l/plate) + NLS-H1 (1.5 μ g/plate)^(3.75)
¹¹/₁₂> DNA + Lipo (2.5 μ l/plate) + NLS-H1 (0.5 μ g/plate)^(1.25) + MAT-H1 (0.5 μ g/plate)^(0.5) (CB pur)
¹³/₁₄> DNA + Lipo (2.5 μ l/plate) + NLS-H1 (0.5 μ g/plate)^(1.25) + MAT-H1 (1.5 μ g/plate)^(1.5) (CB pur)
¹⁵/₁₆> DNA + Lipo (2.5 μ l/plate) + NLS-H1 (1.5 μ g/plate)^(3.75) + MAT-H1 (0.5 μ g/plate)^(0.5) (CB pur)
¹⁷/₁₈> DNA + Lipo " + NLS-H1 (1.5 μ g/plate)^(3.75) + MAT-H1 (1.5 μ g/plate)^(1.5) (CB pur)
¹⁹/₂₀> DNA + " " + NLS-H1 3 μ g/plate^(6.75)
²¹/₂₂> DNA + Lipo " + NLS-H1 4.5 μ g/plate^(10.25)
²³/₂₄> [DNA + NLS-H1 (1.5 μ g)] + DOPE (6 μ l/plate)^(3.75)
²⁵/₂₆> [DNA + NLS-H1 (1.5 μ g)] + MAT-H1 (0.5 μ g) + DOPE (6 μ l/plate)^(3.75)
²⁷/₂₈> [DOPE + NLS-H1 (1.5 μ g)] + DNA (5 μ g/plate)^(3.75)
²⁹/₃₀> [DOPE + NLS-H1 (1.5 μ g)] + MAT-H1 (0.5 μ g) + DNA^(3.75)
³¹/₃₂> DNA 5 μ g/plate + DOPE 6 μ l/plate

10 Day Old Myotubes -

- ¹/₂> DNA (5 μ g/plate) + Lipo (2.5 μ l/plate)
³/₄> DNA " + " " + NLS-H1 (1.5 μ g/plate)^(1.5)
⁵/₆> DNA " + Lipo " + NLS-H1 " + MAT-H1 (0.5 μ g)^(0.5)

* For all samples except 23-32
 - Add DNA (10 μ g/2 plates) in 100 μ l Tris 8.5 + Protein
 ↓ 15' rt
 Add Lipofectin
 ↓ 80' rt

↓
Add to 1.5 ml on each 35mm dish

5

5:28

MEAS. TIME (s) : 31.0

SAMPLE	RLU	
1	543	H ₂ O Bk
2	9204	11 Lucif
3	87503	51 Lucif
4	1436	> 5ug/plate pBSRLUX
5	2385	
6	44422	> DNA + 2.5 μ /plate Lipodectin
7	28742	
8	227053	> DNA + Lipo
9	415636	1.5 μ /plate
10	15875	> DNA + Lipo + NLS-H1 (0.5ug/plate)
11	7391	(2.5)
12	753063	> DNA + Lipo + NLS-H1 + MAT-H1
13	727847	(1.5ug/plate)
14	55082	> DNA + Lipo + NLS-H1 + MAT-H1
15	18582	0.5ug/plate (0.5ug/plate) CB
16	41314	> DNA + Lipo + NLS-H1 + MAT-H1
17	12334	0.5ug (1.5ug/plate) CB
18	18130	> DNA + Lipo + NLS-H1 + MAT-H1 (0.5ug)
19	31812	(1.5ug) CB
20	48416	> DNA + Lipo + NLS-H1 + MAT-H1
21	30253	(1.5ug) (1.5ug) CB
22	2381512	> DNA + Lipo + NLS-H1
23	939243	(2ug/plate)
24	5892126	> DNA + Lipo + NLS-H1
25	3102287	4.5ug/plate
26	182370	[DNA + NLS-H1] + DOPE 60 μ /plate
27	331964	1.5ug
28	225842	[DNA + NLS-H1 + MAT-H1] + DOPE
29	248196	(1.5ug) (0.5ug) CB
30	132754	[DOPE + NLS-H1] + DNA
31	119066	(1.5ug)
32	406366	[DOPE + NLS-H1 + MAT-H1] + DNA
33	240774	CB
34	87183	[DNA + DOPE] 150 μ /plate
35	18896	

* MAT-H1 - purified via
Ni-NTA agarose and then
Cibacron Blue agarose

PC... loaded onto CB column
at 150mM NaCl + eluted
off with 500mM increasing
NaCl step gradient
- checked on 12% SDS-PAGE
- concentrated single band
containing (MAT-H1) elution
- contained 10
- assayed protein (conc)

- Results - Appears that
CB purified protein
inhibits transfectability

* Does it bind & retard
DNA in a gel-shift assay

6/7/94.

43

PS/PE expt #2

1	>	PBSRGYcut ^(3ug)	+	PS 2.5 ^{18λ}	+	NLS-H1 ^(2ug)	8λ	(300 DNA Prot)	+	(300 Liposomes)
2	>	DNA	+	PS 2.5 ^{18λ}	+	"	(4ug)	16λ		
3	>	DNA	+	PS 2.5 ^{18λ}	+	NLS H1	(6ug)	24λ		
4	>	DNA	+	PS 5 ^{18λ}	+	"	(2ug)	8λ		
5	>	DNA	+	PS 5 ^{18λ}	+	"	(4ug)	16λ		
6	>	DNA	+	PS 5 ^{18λ}	+	"	(6ug)	24λ		
7	>	DNA	+	PS 10 ^{18λ}	+	"	(2ug)	8λ		
8	>	DNA	+	PS 10 ^{18λ}	+	"	(4ug)	16λ		
9	>	DNA	+	PS 10 ^{18λ}	+	"	(6ug)	24λ		
10	>	DNA	+	PS 10 ^{18λ}	+	"	(2ug)	8λ		
11	>	DNA	+	PS 10 ^{18λ}	+	"	(4ug)	16λ		
12	>	DNA	+	PS 10 ^{18λ}	+	"	(6ug)	24λ		
13	>	DNA	+	PS 2.5 ^{36λ}	+	"	(4ug)	16λ		
14	>	DNA	+	PS 2.5 ^{36λ}	+	"	(6ug)	24λ		
15	>	DNA	+	PS 5 ^{36λ}	+	"	(4ug)	16λ		
16	>	DNA	+	PS 5 ^{36λ}	+	"	(6ug)	24λ		
17	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
18	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		
19	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
20	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		
21	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
22	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		
23	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
24	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		
25	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
26	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		
27	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
28	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		
29	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
30	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		
31	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
32	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		
33	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
34	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		
35	>	DNA	+	PS 10 ^{36λ}	+	"	(4ug)	16λ		
36	>	DNA	+	PS 10 ^{36λ}	+	"	(6ug)	24λ		

Protocol

- Add DNA + protein (300λ optimum) 15' at rt
- Add Liposomes in (300λ optimum) 15' at rt
- WASH cells 1X in optimum
- Add complex to 2ml optimum on cells
- change media after 3-4 hrs
- incubate at 37°C for ~ 48 hrs
- Harvest cells
- lux assay

3T3

1	1191	RLU	
2	466	> DNA + PS 2.5 + NLS-H1 (18λ) (2ug)	
3	66525	> DNA + PS 2.5 + NLS-H1	
4	107400	18λ 4ug	
5	2181385	> DNA + PS 2.5 + NLS-H1	
6	1997461	18λ (6ug)	
7	485	> DNA + PS 5 + NLS-H1	
8	487	18λ (2ug)	
9	843961	> DNA + PS 5 + NLS-H1	
10	799857	18λ (4ug)	
11	2443514	> DNA + PS 5 + NLS-H1	
12	1974928	18λ (6ug)	
13	686	> DNA + PS 10 + NLS-H1	
14	665	18λ (2ug)	
15	42442	> DNA + PS 10 + NLS-H1	
16	31953	18λ (4ug)	
17	1930695	> DNA + PS 10 + NLS-H1	
18	1779858	18λ (6ug)	
19	366265	> DNA + PS 2.5 + NLS-H1	
20	649356	(36λ) (4ug)	
21	2776187	> DNA + PS 2.5 + NLS-H1	
22	1149081	(36λ) (4ug)	
23	1596987	> DNA + PS 5 + NLS-H1	
24	1792688	(36λ) (4ug)	
25	2789949	> DNA + PS 5 + NLS-H1	
26	3353918	(36λ) (6ug)	
27	270468	> DNA + PS 10 + NLS-H1	
28	236696	(36λ) (4ug)	
29	2482591	> DNA + PS 10 + NLS-H1	
30	2774275	(36λ) (6ug)	
31	2890371	> DNA + PS 10 + NLS-H1	
32	2966735	(36λ) (9ug)	
33	273358	> DNA + Lipofectin	
34	285150		
35		> DNA + Lipofectin	